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**Complete nucleotide sequence of phiCHU: A *Luz24likevirus* infecting *Pseudomonas aeruginosa* and displaying a unique host range**

Damian J Magill<sup>1</sup>, Olga V Shaburova<sup>2</sup>, Elena N Chesnokova<sup>2</sup>, Elena A Pleteneva<sup>2</sup>, Victor N Krylov<sup>2</sup>, Leonid A Kulakov<sup>1</sup>

<sup>1</sup>Queen's University Belfast, School of Biological Sciences, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland

<sup>2</sup>Department of Microbiology, Laboratory for Genetics of Bacteriophages, I.I. Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia

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Correspondence: Leonid A Kulakov, Queen's University Belfast, School of Biological Sciences, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, Tel: +44 (0)28 9097 2799, Fax: +44 (0)28 9097 5877, Email: l.kulakov@qub.ac.uk

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22 **Abstract**

23 A complete nucleotide sequence of the new *Pseudomonas aeruginosa* *Luz24likevirus* phiCHU was obtained. This virus was shown  
24 to have a unique host range whereby it grew poorly on the standard laboratory strain PAO1, but infected 26 of 46 clinical isolates  
25 screened, and strains harboring IncP2 plasmid pMG53. It was demonstrated that phiCHU has single strand interruptions in its  
26 genome. Analysis of the phiCHU genome also suggested that recombination event(s) participated in the evolution of the leftmost  
27 portion of the genome, presumably encoding early genes.

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44 *Pseudomonas aeruginosa* strains are opportunistic pathogens and a significant source of morbidity and mortality (e.g. in cystic  
45 fibrosis patients). In the wake of growing antibiotic resistance, a resurgence of interest in bacteriophage therapy has taken place to  
46 relieve the growing burden observed in healthcare systems (Burns *et al.* 2012; Weinstein *et al.* 2001).  
47 *P. aeruginosa* bacteriophages (phages) are an extremely heterogeneous assemblage and in this work a novel phage of the  
48 *Luz24likevirus* genus, phiCHU, was characterized. PhiCHU was isolated from a small pond in the Moscow locality and shown to

49 have a unique host range; it grew poorly on the standard *P. aeruginosa* laboratory strain PAO1, but infected efficiently a number of  
50 clinical isolates resistant to other phages and lysed mucoid strains isolated from wound infections revealing itself to be a potential  
51 therapeutic agent. How it can circumvent mucoidy is currently unknown. Table S1. shows a comparative analysis of phiCHU's host  
52 range against known lytic phages phiKZ, EL, Lin68, PB1, 14/1, phiKF77, and phiKMV (Mesyanzhinov *et al.* 2002, Hertveldt *et al.*  
53 2005, Krylov *et al.* 2007, Ceyssens *et al.* 2009, Kulakov *et al.* 1986, 1991, Lavigne *et al.* 2003). Of the 46 isolates tested phiCHU  
54 grew well on 26, but exhibited turbid growth and varying plaque morphologies on a number of other strains. Our inability to isolate  
55 lysogens from any strains led us to conclude that phiCHU behaves like a virulent phage, at least in these instances. The  
56 *Luz24likevirus* genus has previously been reported as containing lytic phages apart from PaP3 (Tan *et al.* 2007). PhiCHU's  
57 virulence and broad host range means that it is most likely suitable for use within therapeutic preparations. Importantly, phiCHU  
58 demonstrated good growth on PAO38; a *P. aeruginosa* strain containing the IncP2 plasmid pMG53. Figure S1. demonstrates the  
59 growth of phiCHU along with 8 other phages on *P. aeruginosa* strains. IncP2 plasmids confer a broad spectrum of traits to  
60 pseudomonads including, but not limited to, multiple forms of antibiotic resistance and metabolism of unusual carbon sources  
61 (Jacoby *et al.* 1983). IncP2 being the most abundant plasmids found in nosocomial strains of *P. aeruginosa* (Hanson & Olsen,  
62 1978) were also shown to confer resistance to many phages of *P. aeruginosa* through interference in their intracellular  
63 development. This growth inhibition of different phages has been reported to be under the control of different loci within this plasmid

64 group (Freizon *et al.* 1989). We subsequently investigated whether pMG53 promotes efficient phiCHU growth, by conjugatively  
65 transferring this plasmid to PAO1. Upon carrying this out, PAO1 acquired sensitivity to phiCHU.

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67 The need for phage genomics is paramount from a clinical perspective. Many phages can transfer bacterial genes by transduction,  
68 which poses a significant problem with respect to the potential dissemination of pathogenicity and resistance factors. Apt examples  
69 here would be the *P. aeruginosa* phages E79 and phiKZ (Morgan 1979, Dzhusupova 1982). Genomic analysis can help to elucidate  
70 whether these processes are likely to take place and therefore, whether a given phage is suitable for clinical use. Bacteriophage  
71 phiCHU particles were purified using isopycnic CsCl density gradient centrifugation and genomic DNA was extracted as described  
72 by Sambrook and Russell (2001). The phage was sequenced by the dideoxy method and both shotgun and primer walking on the  
73 whole genomic DNA was employed.

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75 The genomic map of phiCHU is presented in Fig 1. phiCHU was shown to have a linear dsDNA genome of 45,626 bp with a GC  
76 content of 52.02%. 73 ORFs were predicted and annotated in Artemis (Rutherford *et al.* 2000) and functionality was assigned to 22  
77 of these. Five ORFs encode proteins associated with genome replication, recombination and repair, 9 encode various structural  
78 proteins, 3 encode portal and terminase subunits, 2 encode the lysis machinery and the other 2 encode a putative gamma-glutamyl  
79 cyclotransferase and L-Glutamine-D-Fructose-6-Phosphate amidotransferase. All predicted genes lie in two bidirectionally  
80 transcribed units separated by a double intrinsic terminator; a structure indicative of the *Luz24likevirus* genus. Three tRNA genes

81 (tRNA<sup>Asn</sup>, tRNA<sup>Asp</sup>, and tRNA<sup>Pro</sup>) were predicted which are clustered at the extreme right of the genome. The genome is delineated  
82 by 185 bp direct terminal repeats. This genomic organization and a high nucleotide homology of 94.79% to the *Luz24likevirus*  
83 vB\_PaeP\_C1-C14\_Or (Its closest relative) (Accession: HE983844) demonstrates that phiCHU unequivocally belongs to the  
84 *Luz24likevirus* genus of the family *Podoviridae*.

85  
86 Alignments of phiCHU and closely related *Luz24likeviruses* infecting *P. aeruginosa* with the Progressive Mauve algorithm (Darling  
87 *et al.* 2010) highlighted the presence of a gap of approximately 1.5 kb encompassing gp1 – gp4 (Fig. S2). This region shows a  
88 greater homology (94%) to different phages of the group (Luz24 and TL) (Ceyssens *et al.* 2008, Accession: NC\_023583), which  
89 also exclusively infect *P. aeruginosa*. This finding suggests that the phiCHU genome may have evolved as a result of recombination  
90 between ancestors of vB\_PaeP\_C1-C14\_Or and Luz24.

91  
92 Knowledge of the nature of receptors utilized in phage adsorption is important for therapeutic applications. Investigations into tail  
93 fibre proteins can yield useful information with respect to variations in host range. A number of authors have implicated the  
94 presence of glycine rich regions in the identification of tail fibre proteins (Lucchini *et al.* 1999; Nilsson *et al.* 2000; Tetart *et al.* 1998).  
95 20 of the 50 C-terminal residues of ORF 58 are glycines, thereby making this a likely candidate as one constituent of the tail fibre  
96 complex. Variations in this ORF were investigated amongst *Luz24likeviruses* and it was found that phiCHU differed by only a single

97 residue from phiMR299-2 (Alemayehu *et al.* 2012). At this point (residue 267) phiMR299-2 like other *Luz24likeviruses*, possess the  
98 isoteric residues serine or threonine (Fig S3). phiCHU however, possesses proline at this point. Molecular modelling and alignment  
99 of phiCHU and phiMR299-2 putative tail fibres (Fig S4) demonstrated the extent to which this substitution alters protein structure  
100 and therefore, this may represent one mechanism contributing to altered host specificity of phiCHU.

101  
102 It was previously demonstrated that a *P. putida* phage tf, which is distantly related to *Luz24likeviruses* has localized single-strand  
103 interruptions (nicks) in its genome (Glukhov *et al.* 2012). This genomic feature was not previously demonstrated for  
104 *Luz24likeviruses* infecting *P. aeruginosa*. Here it was shown that denatured phiCHU DNA produces multiple bands on agarose gels,  
105 which disappeared upon ligation (Fig. S5a). The use of total genomic DNA as a template in sequencing reactions permitted the  
106 localization of nicks (Fig. 1 and Fig. S5b) and allowed the identification of the consensus associated with this feature (5' –  
107 TACT/RTGMC – 3'). This proved to be the same consensus as previously reported in tf (Glukhov *et al.* 2012). 14 such sites were  
108 reported for tf and 15 for the *P. fluorescens* phage UFV-P2 (Eller *et al.* 2014, Glukhov *et al.* 2012). phiCHU possesses 7 instances  
109 of this consensus. The purpose of this enigmatic feature in phage genomes remains unknown.

110  
111 In this work, a novel member of the *Luz24likevirus* genus of phages infecting *P. aeruginosa* was isolated and characterized.  
112 phiCHU was shown to possess localized single-strand interruptions in its genome. It was also found that this phage exhibited a



113 unique host range, whereby it grew well on PAO1 only in the presence of a plasmid from the IncP2 group. In addition, we found  
114 some evidence suggesting that recombination within disparate members of the genus contributed to the evolution of phiCHU.

115  
116 Genbank Accession Number: KP233880  
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118

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122

## 123 **Supporting Information**

124 Additional supporting information can be found in the online version of this article:

125 **Table S1.** Sensitivity of clinical strains to phages.

126 **Fig S1.** Comparison of the sensitivity of *P. aeruginosa* strains to phages.

127 **Fig S2.** Progressive Mauve alignments of phiCHU with closely related *Luz24likeviruses*.

128 **Fig S3.** Sequence alignment of *Luz24likevirus* putative tail fibre proteins.

129 **Fig S4.** Structural alignment of phiMR299-2 and phiCHU tail fibre molecular models.

130 **Fig S5a.** Visualization of localized single-strand interruptions in phiCHU DNA.

131 **Figure S5b.** Localization of single-strand interruptions through sequencing on whole genomic DNA.

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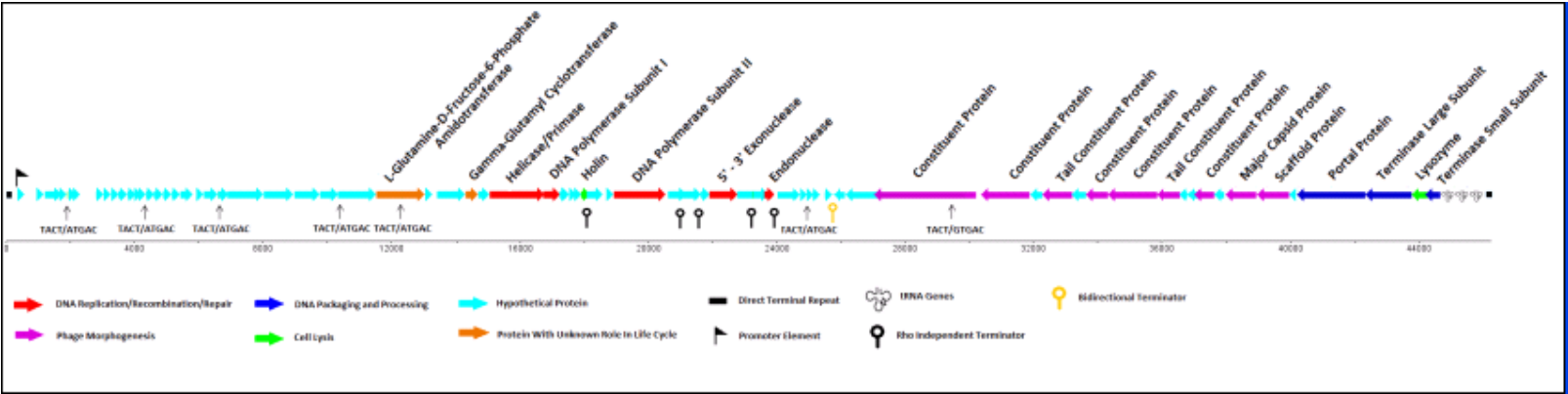
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**Figure Legends**



245

246 **Figure 1. Genome map of the *P. aeruginosa* bacteriophage phiCHU.**

247 Predicted ORFs are displayed as arrows indicating the direction of transcription. Functional annotations (if any) are displayed

248 above ORFs and colour/symbol codes are presented at the bottom of the figure. Sequences associated with localized nicks are

249 displayed at their respective positions